# Notes concerning the development of Nemalion multifidum.

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WITH PLATES XXV AND XXVI.

The resemblance between the structure of the frond and the development of the cystocarp in the genera Batrachospermum and Nemalion is close enough to have led the earlier writers to place them in the same order, and this has also been done by Schmitz in his latest revision of the Florideæ.

Through the researches of Sirodot it has been well established that there is a protonema or chantransia-stage resulting from the germinating spores of Batrachospermum. The surmise in regard to a protonematoid stage in the development of Nemalion also, made by Dr. Farlow in his "Marine Algæ of New England,"[1] has suggested the desirability of making an effort to trace the growth of the spores of this plant. The fortunate discovery of young Nemalion plants, together with an abundance of mature fronds, in the region of Woods Hole, Mass., led the writer during the summer of 1893 to attempt to obtain some further knowledge of this subject.

As far as the writer knows, up to the present nothing in regard to the germination of Nemalion has been published.

The only spores of which we know anything definitely are carpospores. Dr. Farlow quotes Agardh in regard to the presence of tetraspores in the genus Nemalion and says that "no tetraspores have been seen on American specimens of *Nemalion multifidum*."[2] In this same connection it is said by Bornet and Thuret that they have never found tetraspores on any member of the Nemalieæ.[3]

The mature plants of *Nemalion multifidum* Duby consist of slender gelatinous fronds of a deep red-purple color. They often rise with numerous others from a slightly expanded base, which in the older plants is clean and sharply cut, but in the younger is often very irregular in outline. The plants are found attached to rocks or to the shells of barnacles. The

[1] FARLOW, W. G., Marine Algæ of New England 116.
[2] FARLOW, W. G., op. cit. 117.—HARVEY, Nereis Bor. Am. **2**: 134.
[3] BORNET & THURET, Etudes Phycologiques 65.

fronds are from one millimeter to about forty centimeters in length, and from one to three millimeters in diameter. Dichotomous branching is the rule, but irregular branching may be found at various points. Branches occur with more frequency near the tip of the frond where the gelatinous sheath is thinner and where the central filaments can easily make their way to the outside. The writer has never found new branches arising near the very bases of old fronds. The appearance of a tuft of fronds as if all arising from one point, which is frequently seen on barnacle shells, is due to the fact that the young frond sometimes divides very early in its growth into several branches, separated by short intervals, and the expanded base is common to all these branches.

In order to study the earliest development of the Nemalion plants, it was necessary to devise some method of cultivating the spores. For this purpose shallow dishes were used, upon the bottom of which glass slides were placed. A gentle stream of sea-water ran constantly into the dishes, and the water was drawn off from the bottom of each dish over the edge by a siphon. Fronds possessing mature cystocarps were laid over the slides and the spores were shed upon the slides in large numbers. Other spores were collected upon slides placed in dishes which were not supplied with running water. In this case the water was very carefully drawn off and renewed four to five times every day. Spores obtained in this way were watched daily from the 1st until the 12th of August, 1893. The spores, immediately upon being shed, attached themselves to the slides, so that it was possible to keep the same spores under continuous observation, verifying in individuals the changes which were shown in a series of plants. The best results in growth were obtained from the slides which had not been supplied with running water, which was contrary to expectation, since the plants investigated grew on rocks that were exposed to strong buffeting of the waves.

The attached spore is spherical, $12\mu$ to $14\mu$ in diameter, with a stellate chromatophore situated nearly in the center. Bands of granular cytoplasm extend from the cytoplasmic substance surrounding the chromatophore to the peripheral layer of cytoplasm lining the cell wall. There appears to be a double wall to the spore, the inner one thin, while the outer one is firm and thick. Both walls are colorless and transparent. The chromatophore appears as a single body, occupying a large portion of the cell and the deep red color of the whole cell is due to its presence. The nucleus of the spore and of the other cells of the plant is not visible in cells untreated by reagents and hence is not shown in any of the figures of this paper. Figure 1 shows the characteristic form and position of the chromatophore in a freshly attached spore.

The spore itself after remaining apparently unchanged for about twenty-four hours begins to elongate. It extends a protuberance at one end; the chromatophore a little later takes up its position near the tip of this elongating portion and is accompanied by nearly all the cytoplasm of the spore. The protuberance is next separated from the original spore portion by a transverse wall. At this stage, therefore, the sporeling consists of two cells, one a basal almost empty cell, the original spore, and the other, somewhat smaller, ellipsoidal in form (fig. 2). The original spore cell, which loses its chromatophore and a large part of its cytoplasm when the new cell is formed, persists for some time, after gradually losing its contents, as an empty spore-case (figs. 3, 4, 12, 13).

From the newly formed cell there arises in each case, by repeated division, a filament of cells branched or unbranched, each cell of which is similar to the one from which all have originated (figs. 3, 5, 6, etc.).

In many cases branches arise while the sporeling is still very young. Fig. 7 shows the first indication of such a branch in the protrusion of the process *a*. Fig. 8 shows a similar process, *a*, in a later stage, and in fig. 6 there is shown at *a* such a process completely cut off from the parent cell. The branch cell thus formed may contain a chromatophore (fig. 7, *a*), in which case the cell proceeds to divide, forming a series of cells (fig. 9, *a*, *b*), or it may be destitute of a chromatophore, and in this case it develops into a hair. Fig. 6, representing a plant seven days old, shows a typical cell of this sort at *a*.

The sporeling may also form a flat expansion of cells by branches developing in double rows from the original chain of cells as is shown in fig. 9, to which reference has already been made. This bears a decided resemblance to the more advanced structures found at the base of the Nemalion fronds attached to the barnacle shells where they grow. Fig. 10 shows one typical plant of this sort, many of which form red spots on the barnacles, suggestive of the similar spots described by Dr. Farlow as occurring at the base of the fronds of *Mesogloia divaricata* Kütz., which, as also suggested by him, have a definite connection with the growth of the mature frond of that plant.[4]

After the sporeling has developed from three to ten rounded cells, cells of a decidedly different type are developed in the continuation of the filament. These are long, narrow and contain less dense cytoplasmic contents and smaller, less deeply colored chromatophores (figs. 13, 14, 15). In fig. 13, the rounded cells are followed by elongated forms which at *b* are surmounted by two cells which have developed as buds from this last cell of the filament, *b*, and this is the first case of the development of dichotomous branching in the sporeling. From this elementary stage of branching are traced in other sporelings more advanced stages, showing degrees of perfection of dichotomous and of fascicled branching, approaching more and more in their appearance the fascicled branches of the filament of the mature frond, to be described later. Figs. 13, 14, 15 and 12 show such a series, taken from numerous plants of similar structure.

The basal processes of these sporelings are of interest at this point. Fig. 13 shows three of these cells extending back of the spore, *a*. Fig. 14 shows two such processes and three marked branches or rhizoidal processes are found in fig. 15, *a*, *b*, *c*. These are similar to the branched rhizoidal growths formed abundantly at the bases of mature Nemalion plants, and these latter growths are doubtless identical in formation with these sporeling processes.

Fig. 14 is the most interesting form in this series, as the fasciculate branching at its tip shows certain unbranched or single arms, *b*, *b*, *b*, *b*, which, by increase in length, may meet and twist together, forming the elementary condition of a mature frond. These arms would later divide and produce dichotomous fascicles with occasional single arms, which would continue in the manner of the early sporeling to increase in length and thus increase the frond in length and diameter.

In order to throw some light on this very point of the growth of the mature plant in its earliest stage, the growth of the tip of the frond, showing the method of increase in length of the whole plant, was next investigated. The gelatinous mass, which surrounds every filament of the plant, makes it

[4] Farlow, W. G., Marine Algæ of New England 84.

difficult to see the real structure of the tip region. The tip may be seen, however, by directly crushing the frond. This removes too many of the branchlets or disturbs too much their relative position. The application of boiling water to dissolve a large amount of the gelatinous substance allows us to examine the tip with every axial filament and branch in its natural position.

The tip of the plant is never conical. The number of axial filaments and their branches is so great even in the youngest plants examined, that extreme care is necessary to distinguish those occupying the exact tip. It is certain that no single filament at the tip exceeds the others in size, and that there are present at the extreme tip of the plant a number of filaments almost exactly alike (fig. 11). The smallest number of filaments possible to trace to the very tip was three (fig. 11), the lower filaments and the final branchlets of each filament having been gradually removed by gently crushing the plant which had been treated with hot water.

Turning now to the adult frond below the tip, we find that it is made up of distinct axial and cortical layers. Each of these layers is made up of branching filaments. In the axis the filaments are made up of long narrow cells, from 30 to $125\mu$ in length, containing small colorless chromatophores (fig. 11, *a*, *b*). The walls marking the cell divisions of these threads are so far apart (often $125\mu$) as to have made Agardh's statement[5] a most natural one. "The filaments proceeding downwards are inarticulate and cylindrical; those growing upwards are articulated, and more or less contracted at the dissepiments." These central cells divide at irregular intervals, giving off, often without separation by a cell wall, branches which may proceed undivided in the central region or turn towards the periphery, and divide dichotomously (fig. 11, *c*, *d*). Several divisions of a central thread may occur at very short intervals as shown in fig. 11, where five such arms are separated off from the main thread near *e*. Certain of these arms proceed up or down the main axis for a long distance without dichotomous branching, *d*, *f*. Fig. 14 shows the origin of the single arm, as we shall call it, at *b*, *b*, *b*, *b*, together with the other arms or branches which directly form the perfect fascicle. The "downward growing" arms turn to the periphery as much as do the "upward growing" arms, and

[5] HARVEY, Nereis Bor. Am. 134.

form fascicles of branches by dichotomous division (fig. 11, *g*).

Thus tracing these different filaments to the tip, we find at the very last three main axial threads (fig. 11, *1, 2, 3*) bearing lateral fascicles and ending in three distinct fascicles, *4, 5, 6*, each bearing single arms, *7, 8, 9*, like those formed lower down on the frond. These single arms, *7, 8, 9*, by their increase in length will extend upward beyond the divided arms or fascicles of branches, and by later division increase the length of the whole frond.

Increase in diameter is produced by the pushing to the periphery of direct or lateral branches of the axial filaments. These divide dichotomously to form new fascicles (fig. 11, *c, g*, etc.). Very young tufts are thus found with fully fruited ones at the base of even the oldest fronds. Young undeveloped trichophores are found in the next fascicle to one bearing ripe cystocarps. Agardh's description of the mode of the development of the Nemalion frond is interesting, at this point.[6]

Agardh states that the frond consists of three regions, "central, lateral and peripheral." The peripheral branches grow first, and send their branches inward, making the increase in diameter of the frond "like that of an endogenous tree from the outside to the inside."

A definite resemblance between the structure of the tip of the frond and the sporeling tip both in respect to the fasciculate branches shown in detail in figures 12, 13, and 14, and in respect to the single arms or unbranched filaments occurring with these (figs. 14, *b, b, b*, and fig. 11, *7, 8, 9*,) is clearly seen. Both of these conditions have been already described. The possibility of the development of the erect or mature frond from the sporeling plants must now be noticed. The increase in length and subsequent division of any of the filaments of a sporeling plant to form a complete frond has already been mentioned (fig. 14). The meeting of several

[6] "The different strata of the frond seem to me to be formed in an opposite direction. First, unless I am deceived, the peripheric stratum begins to be developed from the base upward by progressive evolution; some of the branches emitted by the peripheric filaments constitute the peripheric stratum, but others are erected with a direction more vertical, giving off on their outer side new peripheric filaments, and on their inward side longitudinal filaments. These latter, at first, by an ob ique course are directed towards the center of the axis; then they take a downward direction, by a longitudinal course. In some respects, therefore, the growth of an endogenous stem is imitated."

such sporeling plants and the consequent interlacing of the filaments belonging to each seems a very probable method of formation for a plant with the axial structure of Nemalion. It also seems probable that the increase in length of the filaments developed on any of the plants found on the barnacle shells which there form red spots (fig. 10), the subsequent division into branchlets, and the interlacing of these filaments may give rise to an erect frond of Nemalion. The possible origin of Mesogloia from similar "spots" has already been mentioned.

In conclusion I would compare or homologize the prostrate series of rounded cells developed from a spore of Nemalion forming a short filament or a flat expansion of cells with that series of prostrate cells formed from the Batrachospermum spore and called by Sirodot a prothallus or protonema, and described by him as such in "Les Batrachospermes." The resemblance seems so exact as to admit of calling this stage in Nemalion also a protonema. From this there arises the branched sporeling already described. The chantransia stage of Batrachospermum is an erect plant, branching irregularly and bearing the sexual plant as a bud. This sexual plant has an axis of single cells placed end to end, covered by the branches which grow up and down its surface. In Lemanea, the chantransia is a similar branching plant, bearing the sexual plant as a bud. The resemblance between this stage in Lemanea and Batrachospermum and the branched sporelings described in this paper is so close as to admit of calling these branched sporelings the chantransia stage of Nemalion.

It remains my pleasant privilege to thank Dr. W. A. Setchell of the University of California for his suggestive direction of the work done on Nemalion at the Marine Biological Laboratory, at Woods Hole, Mass., in 1893, for material collected by him in 1894, and for criticism during the development of these observations.

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## Explanation of Plates XXV and XXVI.

### *Plate XXV.*

Fig. 1. Carpospore of *Nemalion multifidum*. × 400.

Fig. 2. Spore in early stage of germination; three days old. × 400.

Fig. 3. Sporeling in later stage of germination (protonema); three days old. × 400. *a*, empty spore case; *b*, second cell of plant; *c*, colorless protuberance developing into third cell of the plant.

Fig. 4. Another common form of sporeling or protonema; three days old. × 400.

Fig. 5. Characteristic filamentous sporeling or protonema; five days old. × 400.

Fig. 6. Branched protonema with hair cell, *a;* seven days old. × 400.

Fig. 7. Filamentous protonema showing origin of branch, *a;* nine days old. × 400.

Fig. 8. Filamentous protonema with branching process, *a,* further developed; nine days old. × 400.

Fig. 9. Protonema forming flat expansion of cells; ten days old. × 400.

Fig. 10. Protonema with seven branches forming flat expansion on barnacle shell. × 400.

*Plate XXVI.*

Fig. 11. Tip of frond crushed to remove extra filaments and tip branchlets. × 130.

Fig. 12. Detail of typical lateral or tip fascicle of mature frond. × 240.

Fig. 13. Filamentous growth from sporeling showing first indication of dichotomous branching. × 240.

Fig. 14. Older sporeling or "chantransia" stage, with perfect fascicle of branchlets and well developed rhizoidal branches. × 240.

Fig. 15. Chantransia more advanced than the preceding, with several rhizoidal branches. × 240.

⁂ Figs. 1–9 from nature, with Abbé camera. Material under cultivation in 1893.

Fig. 10 from nature, with Abbé camera. Material on barnacle shell.

Figs. 13–15 with Abbé camera. Material cultivated in 1894 by Dr. Setchell and preserved in chrome-alum.